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WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
C12N 5/00, C12P 21/00
C07K 15/00, C12N 15/00
G01N 33/574, 33/577, A61K 49/02
A61K 39/395, 43/00 // (C12P 21/00
C12R 1:91)

A1

(11) International Publication Number: WO 86/ 02945
B24

(43) International Publication Date: 22 May 1986 (22.05.86)

(21) International Application Number: PCT/US85/01511

(22) International Filing Date: 8 August 1985 (08.08.85)

(31) Priority Application Numbers: 670,328
702,059

(32) Priority Dates: 9 November 1984 (09.11.84)
15 February 1985 (15.02.85)

(33) Priority Country: US

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(81) Designated States: AT (European patent), AU, BE (European patent), BR, CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), SU.

Published
With international search report.

(54) Title: MONOCLONAL ANTIBODY TO A HUMAN CARCINOMA TUMOR ASSOCIATED ANTIGEN

(57) Abstract

A murine monoclonal antibody specific to a particular antigenic determinant on the surface or in the cytoplasm of human carcinoma cells and tissue. A process of making said monoclonal antibodies. A cell line is provided for producing such specific monoclonal antibodies specific for the human carcinoma KC-4 tissue antigen and a method of detecting and measuring said antigen. A method of detecting and diagnosing human carinomas by selective labelling of said monoclonal antibodies.

MONOCLONAL ANTIBODY TO A HUMAN CARCINOMA TUMOR ASSOCIATED ANTIGEN

This invention relates to monoclonal antibodies and particularly, to murine monoclonal antibodies which demonstrate reactivity to a specific antigen on the surface or in the cytoplasm of human carcinoma cells and tissue.

The human system involves the production of serum proteins, known as antibodies, by the lymphoid cell series capable of reacting with antigenic determinants which trigger their production. Since the conventional response of the immune system to an antigen with many antigenic determinants is the production of antibodies to each determinant, the antiserum produced is heterologous in nature and polyclonal. or produced by many different cells each producing antibodies to a specific determinant. Antigenic determinants may be referred to as epitopes when more than one occurs on a single molecule and particularly when each elicits an antibody developing. immune response. A single antibody molecule is specific for a unique antigenic determinant or epitope.

Monoclonal antibodies are uniform antibodies directed to a single determinant or epitope on the antigen molecule which may be repeated at several sites of the molecule. Obviously, to produce such monoclonal antibodies <u>in vitro</u> requires selecting a homogeneous antibody having the desired specifications from numerous antibodies elicited in a conventional polyclonal response. The basic technology for <u>in vitro</u> production of homogeneous, highly specific, monoclonal antibodies was developed by Kohler, G. and Milstein, C. (Nature 256:495-497, 1975) known as hybridoma technique. This method involved the immunizing of mice with antigens resulting in the harvesting of antibody-producing cells from those

animals, and fusing these antibody-producing cells with a strain of antibody nonproducing myeloma cells, e.g. plasma cell tumor cells, to produce hybridomas. These hybridomas are robust cells which have all of the in vitro survival and growth stamina of the myeloma cell line and antibody producing quality of the B lymphocytes with which it was fused. The hybridomas thus produce monoclonal antibodies and may either be cultured in vitro or may be grown as tumors in a host animal. Since each antibody-producing cell produces a single, unique antibody, the monoclonal cultures of hybridomas each produce a homogeneous antibody which may be obtained either from the culture medium of hybridoma cultures grown in vitro or from the cells, injected into the peritoneal cavity of mice producing ascitic fluid, or serum of a hybridoma tumor bearing host animal.

Although the general scheme of hybridoma and monoclonal antibody production is well known at this stage of implementation, great care must be exercised in the separation and maintenance of hybridoma cells in culture. Isolated clones have been known to produce antibodies against a subject antigen which differs from clone to clone since antibodies produced by different cells may react with different antigenic determinants on the same molecule. Adequate testing of the resulting antibody or antibody-containing medium, serum or ascitic fluid is essential. It is necessary to characterize the antibody of each clone which contributes to the complexity of producing monoclonal antibodies which are to be utilized in both diagnostic and therapeutic applications.

In developing a desired monoclonal antibody, one must identify and locate the antigenic determinant which will elicit a specific antibody to bind with it. Or, conversely, develop several hundred hybridoma clones from fusions performed and exhaustively screen them

against normal and non-normal tissue and different antigens in identifying and defining that clone which produces the antibody with desired binding specificity. According to this invention the antibody produced detects structural differences on cell surface markers associated with the onset of adenocarcinoma and squamous cell carcinoma, the primary types of carcinoma. The primary object of this invention is to create and maintain hybridomas which produce monoclonal antibodies which will bind with such a particular antigenic determinant to achieve this desired functional specificity.

It is known that monoclonal antibodies may be labeled with a selected variety of labels for desired selective usages in detection, diagnostic assays or even therapeutic applications. In each case, the binding of the labelled monoclonal antibody to the determinant site of the antigen will signal detection or delivery of a particular therapeutic agent to the antigenic determinant on the non-normal cell. A further object of this invention is to provide the specific monoclonal antibody suitably labelled for achieving such desired selective usages thereof.

This invention has particular application to achieving identification of carcinoma cells which occur in the specific diseases of adenocarcinoma and squamous cell carcinoma, the primary forms of carcinoma.

Murine monoclonal antibodies specific to a unique antigenic determinant on the surface and in the cytoplasm of human neoplastic tissue are produced. The unique antigenic determinant is designated the "KC-4 antigen" which is capable of eliciting an antibody which binds selectively only to neoplastic carcinoma cells and not to normal human tissues. The unique antigen appears in two forms in carcinoma cells of which only the smaller is expressed in the cell membrane. The first is the larger

form and appears only in the cytoplasm and has a molecular weight of approximately 490,000 daltons (range of 480,000 - 510,000). The second form occurs at higher density expression and is found in both the cytoplasm and membrane of carcinoma cells and has a molecular weight of approximately 438,000 daltons (range of 390,000 - 450,000) determined by subjecting the KC-4 antigen to electrophoresis methodology and comparing movement thereof with market protein molecules of known molecular weight (Towbin, et al Proc. Natl. Acad. Sci. 76:4350-4354, 1979 and Laemmli, U.K. Nature, 227:680, 1970). The monoclonal antibody, called "KC-4" of the invention has useful application in the areas of diagnosis and medical treatment of a plurality of carcinomas by means of selective labels affixed thereto.

The KC-4 monoclonal antibody is particularly useful in its application to binding with the antigenic determinants on and in carcinoma cells which occur in the specific diseases of adenocarcinoma and squamous cell carcinoma regardless of the human organ of origin.

The present invention provides murine monoclonal antibodies specific to a particular antigen on the surface or in the cytoplasm of human carcinoma tissue, such as adenocarcinoma and squamous cell carcinoma. This unique antigen, designated "KC-4 antigen", was developed from human carcinoma tissue involving prostate adenocarcinoma. All monoclonal antibodies having this specificity for the defined "KC-4 antigen" can be referred to as "KC-4".

A Balb/c mouse was innoculated intraperitoneally over a two week period using an initial injection of prostatic adenocarcinoma cells. Two additional injections followed using as an immunogen a crude tumor homegenate from the

same tumor. The spleen of the mouse was perfused four days following the additional injections to isolate individual cells. Then, cells of the mouse plasmacytoma cell line, known as Sp2/O-Ag14, were fused with the mouse splenocytes using a modified Kohler and Milstein procedure (Nature 256:495-497,1975). Fused cells were then cultured for 10-14 days in HAT media to develop cell colonies capable of multiplying in the media. Conditioned media containing the antibody secreted from each colony was removed and screened for specific activity. Media was used to stain normal and prostatic adenocarcinoma tissue. Fused cell colonies exhibiting the desired reactivity were single cloned and further tested on a variety of normal and neoplastic tissues including carcinoma.

The cloning procedure for the selected fused cell colonies, which were KC-4 producing colonies, was performed in soft agar. Cells were mixed with liquified agarose and the mixture was plated in well plates and allowed to solidify. Then, the plates were incubated and monitored, individual clones being harvested between 10 to 14 days. The individual clones were each screened by immunoperoxidase and immunoflourescent staining of human tissue and cell lines. Clones producing the desired antibody were isolated and cloned again in agarose to further assure stability and monoclonal nature.

The monoclonal antibody "KC-4" demonstrates an intense membrane and cytoplasmic antigen distribution on carcinoma cells and gave no specific or positive staining pattern on normal human tissue.

Reactivity of the KC-4 monoclonal antibody on normal and neoplastic human tissues was determined using two methods including biotin/avidin immunoperoxidase and immunofluorescence staining procedures. Both fixed and paraffin embedded tissue, frozen sections, fresh tumor cells and cell lines were used to demonstrate tissue

distribution of the specific antigen being identified. A positive result with KC-4 is seen as an intense membrane and/or cytoplasmis cytoplasmic A neoplastic specimen showed positive staining of the majority of tumor cells present. No specific reactivity with normal tissue specimens or normal cells has been observed throughout the screening analyses.

One hundred and four different cases of solid tumors or lung, colon, kidney, breast, stomach, prostate, pancreatic, lymph node ductal, and lymphoma different tumor tissues were tested with the KC-4 antibody. All such cases were heat processed, paraffin prepared tissues. Ninety-four percent of these cases (98/104) were positive. All positive staining appeared only on tumor cells while all normal tissue remained unaffected. The six percent false negative staining was attributed to poorly prepared tissue which destroyed rather than preserved KC-4 expression.

Ninety-two different cases of paraffin embedded normal tissue including spinal cord, breast, uterus, thyroid, tongue, prostate, spleen, adrenal, lung, kidney, gall bladder, heart, lymph node, stomach, colon, liver, brain, testes, thymus, and placenta were tested with the KC-4 antibody. All 92 cases were heat processed, paraffin prepared tissues. Only 15.2% (14/92) demonstrated some staining. In all of these positives, the staining was attributed to normally occurring artifacts found in these tissues. The greatest amount of non-specific staining of the normal tissue was in breast, kidney, and stomach tissue. The staining in the breast tissue was found in the alveolar cells of the glands. This is a common finding and is considered to be nonspecific on the antibody. The convoluted distal tubules picked up some staining in the kidneys. This is seen with almost all antibodies and is non-specific in origin. Mucous picks up

the stain with most antibodies and this is the case with the normal stomach tissue and KC-4. This staining is considered non-specific and artifactual.

Thirty-three different normal tissues from prostate, lung, kidney, liver, lymph node, spleen, colon, thymus, breast, gall bladder and stomach were processed by fresh frozen section and tested with the KC-4 antibody. No heat was used in processing these specimens. Only 3% (1/33) demonstrated any positive staining. It should be noted that frozen tissue sections are more like the fresh tissue than heat processed, formalin fixed, and paraffin embedded tissue. Therefore, the difference is percent positive staining of KC-4 on normal frozen tissue (3%) versus normal fixed/embedded tissue (15%) is artifactually created in the method of tissue preparation.

Further analyses were conducted on frozen human tumor tissue of colon, prostate, lung, and breast carcinoma with KC-4 antibody staining. One hundred percent of the neoplastic carcinoma tissues were positive with KC-4 i.e., deep cytoplasmic and cell surface specific staining was observed.

The KC-4 antigen molecule was isolated and identified as having two forms. The larger of the forms has an approximate molecular weight of 490,000 daltons (range of 480,000 - 510,000) and occurs only in the cytoplasm of carcinoma cells. The smaller form has an approximate molecular weight of 438,000 daltons (range of 390,000 450,000) and occurs in both the cytoplasm and the membrane of carcinoma cells. This isolation was accomplished by lysing cells of the HT-17 cell line, derived from a human breast carcinoma, in distilled water at 1 x 108 cells/ml employing repeated freezing and thawing. The lysates were centrifuged at 100,000 x g to prepare a membrane pellet and a cytoplasm supernatant. The cytoplasm was diluted 1:1 in SDS-PAGE sample buffer. The membranes were

dissolved in SDS-PAGE sample buffer. Both samples were heated to 90° for 5 minutes. Subsequently, 23 x 10^6 cells equivalent of each sample was run on SDS polyacrylamide (3.5 - 10% gradient) electrophoresis carried out on a discontinuous vertical slab gel according to a modification of the procedure described in Laemmli, U.K. Nature 227,680,1980. The internal molecular weight markers were fibrinogen (340,000), fibronection (440,000), myosin (200,000), beta-galactosidase (116,000), phosphorylase B (92,500), bovine Albumin (66,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and alpha-lactalbumin (14,000). After electrophoresis, the proteins in the acrylamide slab were electroblotted to a sheet of nitrocellulose according to a modification of the procedures described in Towbin (1979) Proc. Natl. Acad. Sci., 76,4350. The nitrocellulose was then blocked in bovine albumin containing buffer. Monoclonal antibody, KC-4, was then reacted with the nitrocellulose to bind to the specific antigen located on the nitrocellulose. After washing away unbound KC-4 antibody, an anti-mouse immunoglobulin, enzyme conjugate was reacted with the KC-4 antibody bound to the nitrocellulose. After washing away unbound conjugate, enzyme substrate was added and colored bands appear where the KC-4 antigen had migrated.

The "KC-4" monoclonal antibody specifically reactive with the KC-4 antigen was found in two forms. A mouse IgG3 isotype and an IgM as evidenced by its reactivity with a goat anti-mouse IgG3 and IgM antibody and its lack of reactivity with other goat and/or rabbit anti-mouse immunoglobulin isotype specific antibodies.

A sample of both hybrid cell lines capable of producing monoclonal antibodies specific for the KC-4 antigen are on deposit with the American Type Culture Collection and are assigned the Nos. HB 8709 (IgG3) and HB 8710 (IgM).

The availability of homogeneous, highly specific

monoclonal antibodies is an especially valuable tool for diagnostic and therapeutic applications in the detection and treatment of human carcinomas.

As a diagnostic tool, the KC-4 monoclonal antibodies can be brought into contact with a biological sample of human carcinoma cells derived from human neoplasia. Immunological complexes derived between the monoclonal antibody and carcinoma cells in the biological sample can be detected, said complexed cells being monoclonal antibody and human neoplastic cells.

This methodology can also be applied to detect and measure the KC-4 antigen in serum or other liquid biological samples derived from human patients suspected of having human carcinoma or related tumors.

Further, said complexes can be detected by contacting that biological sample of the human carcinoma with a second antibody capable of binding to the KC-4 monoclonal antibody. Said second antibody is labeled with a detectible compound (detector group) selected to enable said complexes to be labelled with said detectible compound when said second antibody binds to said monoclonal antibody specific for the KC-4 antigen. The resulting labelled complex can then be detected. For diagnostic applications, said detector group can be selected from a fluorescent compound, an enzyme which produces absorptive or fluorescent detector group when reacted with a specific substrate, radioactive element, or an electron dense compound. (Goldman, Morris Fluorescent Antibody Methods, Academic Press, New York, 1968; Yoshitake, S. et al. Scand. J. Immunol. 10:1-6, 1979; Hunter, W.M. & Greenwood, F.C. Preparation of iodine 131 labeled growth hormone of high specific activity. Nature 194,495,1962).

Detector groups suitable for this function include fluorescent compounds such as fluorescein, rhodamine,

phycoerythrin, cyamine dyes, and any other compound emiting fluorescence energy Other catagories of detector groups include enzyme substrate products which form fluorescent compounds such as N-methylumbelliferone-B-D-galactosidase or absorptive compounds as DAB (di-aminobenzidine). There are many others in these categories. Radioactive elements which are suitable as detector groups include Iodine-125, Iodine-131, Indium 11 Bismith-210, and several others of which these are presently the most often used compounds. Electron dense detector groups would include such compounds as gold and ferric chloride, as presently known. Although this approach is predominately employed on in vitro diagnostic applications it does not exclude in vivo diagnostic or therapeutic application of similarly labeled KC-4 antibody.

The KC-4 monoclonal antibody can be used for detecting carcinoma in a human patient. In this application, KC-4 monoclonal antibody is treated to develop a label thereon capable of producing a detectible signal and infusing said monoclonal antibody into the patient thereby labeling said tumor when the monoclonal antibody binding to the antigenic determinant thereof. Such a detectible label can comprise a radioactive element, a fluorescent compound or other suitable detectible label or compound. This approach is equally suited for in vitro diagnostic detection of carcinoma cells on tissues which have been frozen, fixed, or fixed and heat processed with paraffin embedding. Additional in vitro applications include the radioimmunoassay or radioimmunometric assay or enzyme immunoassay or nephlemetric detection of KC-4 antigen in serum, plasma, or other liquid based biological samples such as cerebral spinal fluid, urine, and sputum.

For therapeutic treatment with the intent of inhibiting or eliminating human carcinoma in a patient

suspected of having such a tumor, the KC-4 monoclonal antibody of KC-4 conjugated with a suitable toxic agent can be injected into the patient in a controlled protocol of administrations whereby said monoclonal antibody or monoclonal antibody--toxic agent-conjugate can bind to the tumor and effect tumor cell death. Examples of such a toxic agent can be a chemotherapeutic agent, a photo-activated toxic agent or radioactive agent. Examples of such a radioactive agent are Iodine-125, or Bismith-210. Examples of a chemotherapeutic agent would include the alpha chain or A-chain ricin, diphtheria, or whole molecules, cytoxin adriamycin, methyltrexate, and platinium compounds, such as cisplatin. Examples of photo activated toxic agents include infrared dyes, such as in the cyanine family.

Claims:

1. A hybrid cell line which produces a monoclonal antibody specific for the human carcinoma KC-4 tissue antigen.

2. The cell line according to claim 1 characterized in that said antibody-producing cell is derived from the murine genus.

3. The cell line according to claim 1 characterized in that said antibody-producing cells are mouse spleen cells.

4. The cell line according to claim 3 characterized in that said cells are derived from mice immunized with human carcinoma cells.

5. The cell line according to claim 1 characterized in that said cells are derived from a fusion of mouse myeloma cells.

6. The cell line of claim 3 characterized in that the antibody-producing cells were derived from Balb/c mice.

7. The hybrid cell line of any one of claims 1 to 6 characterized by the identifying characteristics of the sample on deposit with the American Type Collection Nos. HB8709 (IgG3) or HB8710 (IgM).

8. The cell line of any one of claims 1 to 7 characterized in that said KC-4 antigen is sited on the surface or in the cytoplasm of the human carcinomas and the antigen further is characterized as having an approximate molecular weight of 438,000 daltons (range of 390,000 - 450,000) and 490,000 daltons (range of 480,000-510,000) as determined by electrophoresis methodology applied to the antigen and comparing the antigen movement with that of known marker proteins of known molecular weights.

9. A monoclonal antibody specific to a particular antigen on the surface or in the cytoplasm of human carcinoma tissue, said antigen being further

characterized in that

a. it has an approximate molecular weight of 390,000 - 450,000 as determined by carrying out electrophoresis on the antigen and comparing its movement with that of marker proteins of known molecular weight.

b. it may be expressed in a slightly larger form found only in the cytoplasm having an approximate molecular weight of 490,000 daltons (range of 480,000 - 510,000) as determined by said electrophoresis method.

c. it is not expressed specifically on normal tissue, and

d. it is not modulated by a human carcinoma cell line.

10. The monoclonal antibody of claim 9 which is produced by the hybrid cell line having the identifying characteristics of ATCC HB8709 (IgG3) and HB8710 (IgM).

11. A method of detecting or measuring human carcinoma cells derived from human carcinoma in a biological sample, said method characterized by the steps of 1) contacting said biological sample with a monoclonal antibody specific to a particular antigen on the surface of or in the cytoplasm of human carcinoma tissue, said antigen having an approximate molecular weight of 438,000 daltons (range of 390,000 - 450,000) as determined by carrying out electrophoresis on the antigen and comparing its movement with that of marker proteins of known molecular weight capable of being expressed in a slightly larger form found only in the cytoplasm having an approximate molecular weight of 490,000 daltons (range of 480,000 - 510,000) as determined by said electrophoresis method, not expressed specifically on normal human tissue, and

not modulated by a human carcinoma cell line and 2) then detecting immunological complexes formed between said monoclonal antibody and cells in said sample, the cells which are complexed with said antibody being human carcinoma cells.

12. The method of claim 11 wherein said method is used to detect or measure human carcinoma cells in a biological sample obtained from a human patient suspected of having such a carcinoma tumor.

13. The method of claim 12 characterized in that the step of detecting of said complexes includes the steps of 1) contacting said biological sample with a labelled second antibody capable of binding to said monoclonal antibody, said second antibody being labelled with a detectible compound such that said complexes are labelled with said detectible compound when said second antibody binds to said monoclonal antibody, and 2) detecting said labelled complexes.

14. The method of claim 13 characterized in that said detectible compound is a fluorescent compound.

15. The method of claim 13 characterized in that said detectible compound is an enzyme which produces said detectible compound.

16. The method of claim 13 characterized in that said detectible compound is a radioactive element.

17. The method of claim 13 characterized in that said detectible compound is an electron dense element.

18. A method of detecting human carcinoma in a patient suspected of having such a tumor, said method characterized by the step of infusing KC-4 monoclonal antibody derivitized with a radioactive element into said patient, thereby labelling said tumor with the detectible radioactive element.

19. A method of inhibiting or eliminating human carcinoma in a patient suspected of having such a

tumor, said method characterized by the step of infusing KC-4 monoclonal antibody or a said KC-4 monoclonal antibody-toxic agent conjugate into said patient thereby contacting said tumor and causing tumor cell death.

20. The method of claim 19 characterized in that said monoclonal antibody is administered to said patient in a series of more than one administration.

21. The method of claim 19 characterized in that said toxic agent is a chemotherapeutic agent.

22. The method of claim 19 characterized in that said toxic agent is a photoactivated toxic agent.

23. The method of claim 19 characterized in that said toxic agent is a radioactive agent.

24. The method of claim 23 characterized in that said radioactive agent is Iodine 125 or Bismith 210.

25. The method of claim 19 characterized in that said toxic agent is selected to effect lysing of the tumor cell with which the antibody binds.

26. The method of claim 25 characterized in that said toxic agent is selected from either animal complement used in lysing cells in vivo or in vitro.

27. A murine monoclonal antibody of the mouse IgG3 or IgM isotype which is specific for the KC-4 antigen.

28. The antibody of claim 27 characterized in that the KC-4 antigen is selected from the surface or in the cytoplasm of certain human carcinoma.

29. The antibody of claim 27 characterized in that it is in detectibly labelled form.

30. The antibody of claim 29 characterized in that said label is one of the following: a fluorescent compound, an enzyme which produces said label, a radioactive or an electron dense element.

31. The antibody of claim 29 characterized in that said label is a chemotherapeutic, photo-activated toxic or radioactive agent.

32. A method of detecting the KC-4 antigen of human carcinoma cells by effecting either agglutination of KC-4 monoclonal antibody coated microspheres with carcinoma cells or KC-4 coated fluorescent microspheres satelliting carcinoma cells.

33. A method as described in claim 32 characterized in that said KC-4 antigen is on the surface of the carcinoma cell.

34. A method as described in claim 32 characterized in that said KC antigen is in a liquid biological samples.

35. A method of detecting and measuring KC-4 antigen in a liquid biological samples characterized by the use of KC-4 monoclonal antibody conjugated with a detector group selected from a fluorescent compound, a radioactive element, or enzyme capable of producing a substrate reaction detectible product.

36. A process of making the hybrid cell line as claimed in any one of claims 1 to 8.

37. A process of making the monoclonal antibody as claimed in claims 9 or 10.

38. A method of making a murine monoclonal antibody of the mouse IgG3 or IgM isotype which is specific for the KC-4 antigen.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 85/01511

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 N 5/00; C 12 P 21/00; C 07 K 15/00; C 12 N 15/00; G 01 N 33/574; G 01 N 33/577; A 61 K 49/02; A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
IPC[4]	C 12 N C 12 P A 61 K G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	EP, A, 0087898 (CARLTON MEDICAL PRODUCTS LIMITED) 7 September 1983, see page 4, lines 8-12; page 8, lines 11-19; page 9, lines 1-21; page 11, line 23 - page 12, line 6; page 40, lines 4-13; claims 1,6 --	1-17,27-38
X	Chemical Abstracts, volume 102, nr. 13, 1 April 1985, Columbus, Ohio, (US) P.W. Andrews et al.:"Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells", see page 516, abstract nr. 111114j & Hybridoma 1984, 3(4), 347-61 (Eng) --	1-17,27-38
X	Biological Abstracts, volume 73, 1982, Philadelphia, (US) B.S. Wilson et al.:"Distribution and molecular characterization of a cell surface and a cytoplasmic antigen detectable in human melanoma cells with monoclonal antibodies",	

* Special categories of cited documents: **

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
	04 DEC. 1985
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 85/01511

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 43/00 // (C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [5]

Classification System	Classification Symbols
IPC[4]	

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [6]

Category [7]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No [13]
	abstract nr. 48110 & Int. J. Cancer 28(3): 293-300, 1981 --	1-17,27-38
A	Biological Abstracts, volume 79, 1985, Philadelphia, (US) K.S. Webb et al.:"Characterization of prostate tissue directed monoclonal antibody", see abstract nr. 51224 & Cancer Immunol Immunother 17(1): 7-17, 1984 --	1-17,27-38
A	Biological Abstracts, volume 75, 1983, Philadelphia, (US) J.J. Starling et al.:"Monoclonal antibodies to human prostate and bladder tumor-associated antigens", see abstract nr. 66094 & Cancer Res 42(8): 3084-3089, 1982 --	1-17,27-38
A	US, A, 4172124 (H. KOPROWSKI) 23 October 1979, see column 1, lines 35-56; claims 1-7,11-14	1-17,27-38

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th November 1985	04 DEC. 1985
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 18-36 because they relate to subject matter not required to be searched by this Authority, namely:

SEE RULE PCT 39.1. iv:

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers ____ because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out specifically

3. ☐ Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a)

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 85/01511 (SA 10478)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/11/85

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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